

CLAIMS

1. A method for releasing a soluble or membrane associated intracellular protein of interest (POI) from a cell comprising the steps of:

- 2645 (a) providing a cell comprising a soluble or membrane associated intracellular POI;
- (b) contacting the cell with a membrane extracting composition comprising a quarternary ammonium compound; and
- (c) causing the POI to be released from the cell under conditions sufficient for the specific release of the POI and in a soluble form.

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2. The method according to claim 1, wherein the quarternary ammonium compound is selected from the group consisting of Lauroyl Trimethyl Ammonium Bromide (LTAB), Myristyl Trimethyl Ammonium Chloride (MTAC), CetylTrimethyl Ammonium Chloride (CTAC), Cetrimide, Cetyl Trimethyl Ammonium Bromide (CTAB), Stearoyl

2655 Trimethyl Ammonium Chloride (STAC), Stearoyl Trimethyl Ammonium Bromide (STAB), Benzalkonium Chloride (alkyldimethylbenzylammonium chloride), N-Cetylpyridinium Bromide (N-Hexadecylpyridinium bromide), N-Cetylpyridinium Chloride (N-Hexadecylpyridinium chloride), Benzyl Dimethyl Tetradecyl Ammonium Chloride, Benzyl Dimethyl Hexadecyl Ammonium Chloride and a combination of any two or more thereof.

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3. The method according to claim 1, wherein the membrane extracting composition comprises from about 0.05% to about 0.6% by weight of the quarternary ammonium compound.

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4. The method according to claim 3, wherein the membrane extracting composition comprises from about 0.1% to about 0.5% by weight of the quarternary ammonium compound.

5. The method according to claim 4, wherein the membrane extracting

2670 composition comprises from about 0.2% to about 0.45% by weight of the quarternary

ammonium compound.

6. The method according to claim 5, wherein the membrane extracting composition comprises about 0.4% by weight of the quarternary ammonium compound.

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7. The method according to claim 1, wherein the cell is contacted with the membrane extracting composition at temperatures from about 4°C to 40°C.

8. The method according to claim 7, wherein the cell is contacted with the
2680 membrane extracting composition at temperatures from about 20°C to about 30°C.

9. The method according to claim 8, wherein the cell is contacted with the membrane extracting composition at temperatures from about 25°C.

10. The method according to claim 1, wherein the cell is contacted with the
2685 membrane extracting composition at a pH optima of from about 2.0 to about 11.0.

11. The method according to claim 10, wherein the cell is contacted with the membrane extracting composition at a pH optima of from about 5.0 to about 7.0.

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12. The method according to claim 11, wherein the cell is contacted with the membrane extracting composition at a pH optima of from about 6.3.

13. The method according to claim 1, wherein the cell is selected from the group
2695 consisting of yeast cells, fungal cells and bacterial cells, preferably from yeast and fungal cells.

14. The method according to claim 13, wherein the cell is selected from the group consisting of yeast cells and fungal cells.

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15. The method according to claim 1, wherein the cell is a transformed cell.

16. The method according to claim 1, wherein the cell is transformed with a nucleic acid encoding the POI.

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17. The method according to claim 1, wherein the intracellular POI is produced by recombinant DNA techniques.

18. The method according to claim 1, wherein the POI is an IL-1ra enzyme.

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19. The method according to claim 1, wherein the POI is a glucan lyase enzyme.

20. The method according to claim 19, wherein the yield of glucan lyase is 1 g/litre or more.

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21. The method according to claim 19, wherein the yield of glucan lyase is 3.5 g/litre or more.

22. The method according to claim 1, wherein the POI is a HOX enzyme.

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23. The method according to claim 22, wherein the HOX enzyme comprises the amino acid sequence set out in SEQ ID No 22 or a variant, homologue, derivative or fragment thereof.

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24. The method according to claim 22, wherein the HOX enzyme is encoded by a nucleotide sequence set out in SEQ ID No 22 or a variant, homologue, derivative or fragment thereof.

25. The method according to claim 22, wherein the HOX enzyme is encoded by a nucleotide sequence capable of hybridising to the nucleotide sequence set out in SEQ ID No

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22 or a variant, homologue, derivative or fragment thereof or a sequence complementary to the hybridisable sequence.

26. The method for screening for mutated cells or transformed cells producing
2735 elevated levels of a soluble or membrane associated intracellular POI comprising the steps of:

- (a) growing the mutated cells at 30°C;
 - (b) incubating the mutated cells or transformed cells with the membrane extracting composition comprising a quarternary ammonium compound,
 - 2740 (c) recovering the cell free medium; and
 - (d) screening the cell free medium for elevated levels of the intracellular POI;
- such that the presence of the intracellular POT in the cell free medium is indicative that the intracellular POI has been released.

2745 27. A membrane extracting composition suitable for specifically releasing a soluble or membrane associated intracellular POI, wherein the composition is contacted with the cell under the following conditions:

- (a) a percentage by weight of quarternary ammonium compound from about 0.05% to about 0.6%;
 - 2750 (b) a pH optima of from about 2.0 to about 11.0; and
 - (c) a temperature optima of from about 4°C to about 40°C;
- such that the intracellular POI substantially free of contaminating proteins is obtained.

28. A method of using a membrane extracting composition comprising a
2755 quarternary ammonium compound to selectively release a soluble or membrane associated intracellular POI.

29. A HOX enzyme producible by a method according to claim 1, wherein the HOX enzyme is encoded by a nucleotide sequence set out in SEQ ID No 22 or a variant,
2760 homologue, derivative or fragment thereof, or a sequence complementary to the hybridisable

sequence, and wherein the nucleotide sequence is synthesised by the oligonucleotides as set out in SEQ ID Nos 2-22.

30. A POI as defined in claim 1, wherein the POI is released in a substantially non-
2765 glycosylated form from a eukaryotic host organism.

31. A substantially non-glycosylated POI released from a eukaryotic host
organism.

2770 32. A substantially non-glycosylated POI released from a eukaryotic host
organism, wherein the POI is released by the method of claim 1.